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When do myopia genes have their effect? Comparison of genetic risks between children and adults

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ABSTRACT

Purpose: Previous studies have identified many genetic loci for refractive error and myopia. We aimed to investigate the effect of these loci on ocular biometry as a function of age in children, adolescents and adults.

Methods: The study population consisted of three age-groups identified from the international CREAM consortium: 5,490 individuals aged <10 years; 5,000 aged 10-25 years; and 16,274 aged >25 years. All participants had undergone standard ophthalmic examination including measurements of axial length (AL) and corneal radius (CR). We examined the lead SNP at all 39 currently known genetic loci for refractive error identified from genome-wide association studies (GWAS), as well as a combined genetic risk score (GRS). The beta coefficient for association between SNP genotype or GRS versus AL/CR was compared across the 3 age groups, adjusting for age, sex, and principal components. Analyses were Bonferroni-corrected.

Results: In the age-group <10 years, 3 loci (*GJD2*, *CHRNA1*, *ZIC2*) were associated with AL/CR. In the age-group 10-25 years, 4 loci (*BMP2*, *KCNQ5*, *A2BP1*, *CACNA1D*) were associated; and in adults 20 loci were associated. Association with GRS increased with age; $\beta = 0.0016$ per risk allele ($P = 2E-08$) in <10 years, 0.0033 ($P = 5E-15$) in 10-25 year-olds, and 0.0048 ($P = 1E-72$) in adults. Genes with strongest effects (*LAMA2*, *GJD2*) had an early effect that increased with age.

Conclusion: Our results provide insights on the age span during which myopia genes exert their effect. These insights form the basis for understanding the mechanisms underlying high and pathological myopia.

Key words: myopia, genetic risk, development, SNPs

INTRODUCTION

The prevalence of myopia (nearsightedness) has increased dramatically in developed countries in recent decades [Bar Dayan, et al. 2005; Vitale, et al. 2009]. Myopia is a complex, multifactorial disease with increasing public health burden due to a strong rise worldwide. In particular high myopia is associated with blinding complications such as myopic macular degeneration, glaucoma and retinal detachment [Curtin and Karlin 1971; McBrien and Gentle 2003; Saw 2006]. High myopia mostly has its onset in early childhood before age 10 years [Fledelius 2000].

The eye's dimensions alter markedly during the peak development phase between birth and the late teenage years, ultimately exerting very strong effects on final refractive error (RE) in later adult life. A complex process called emmetropisation aims to coordinate ocular development, bringing light into clear focus on the retina. Early life myopia is characteristically associated with excessive axial length (AL) increase. This results in a mismatch of the optical effects of the various refractive components of the eye, resulting in a focal point in front of the retina. Such a mismatch can be described by the ratio of AL to corneal radius (CR), AL/CR ratio, which has a high correlation with RE [Hashemi, et al. 2013; Ip, et al. 2007] and is independent of cycloplegia which may vary between studies.

Various studies have examined the heritability of myopia showing increased risk for first-degree relatives of affected individuals [Farbrother, et al. 2004; Guggenheim, et al. 2000] and twins [Sanfilippo, et al. 2010; Young, et al. 2007]. Numerous genetic loci that cause familial high myopia (*MYP1-18*) have been discovered using linkage analysis [Baird, et al. 2010]. More recently, genome wide association studies (GWAS) in large cohorts have been performed to identify further determinants for REs in the general population. The first single nucleotide polymorphisms (SNPs) identified were near *GJD2* [Solouki, et al. 2010] and *RASGRF1* [Hysi, et al. 2010]. Later many more loci were found in studies of large populations (CREAM; 23andMe)[Kiefer, et al. 2013; Verhoeven, et al. 2013] [Wojciechowski and Hysi 2013].

All previously published refractive error GWAS studies were performed in cohorts enrolling participants aged 25 years and older. We aimed to study the effect size of the 39 GWAS-identified genetic regions associated with refractive error to date, as a function of age.

METHODS

Study specific analysis

We included 18 cohorts from 8 different countries in Europe, Asia and Oceania, with a total of 5,490 children <10 years, 5,000 individuals of 10-25 years, and 16,274 adults, all with phenotypic and genome-wide genotypic data available. Age cut off points were based on prior knowledge regarding eye growth. The eye has the highest growth rate before the age of 10 years, and generally does not grow in axial length after age 25 years [Zadnik, et al. 2003]. Details on subject recruitment procedures can be found in the supplemental materials. Each study participant was genotyped with either an Affymetrix or Illumina SNP array (supplemental table I). All studies were conducted according to the Declaration of Helsinki. The studies were approved by the local review boards. Written, informed consent for the collection and analysis of measurements of all study participants was obtained.

SNPs

A total of 39 SNPs were included in this analysis. The SNPs were selected based on their known association with RE and myopia in the GWAS carried out by CREAM [Verhoeven, et al. 2013] and 23andMe [Kiefer, et al. 2013](supplementary table II). An unweighted genetic risk score (GRS) was calculated for each participant by summing the dosage of risk alleles (scale 0-2) for all 39 SNPs. The risk score was normally distributed.

Ocular biometry

The ocular biometry measurements included AL and CR, and the AL/CR ratio was calculated. Multiple measurements of AL and CR were taken of the right eye and left eye, were averaged to calculate a mean AL and CR for each eye. The average AL of both eyes was divided by the

average CR of both eyes to calculate the AL/CR ratio. Details of the phenotypic assessment protocols/instruments used in each study can be found in the supplemental material.

Meta-analysis

All studies performed linear regression models with each SNP or the GRS as determinants, and the AL/CR ratio as outcome. Analyses were adjusted for the potentially confounding effects of age and gender, and additionally – to account for ancestry differences within the sample – for principal components where applicable. A meta-analysis was performed to estimate the beta effects using an inversed variance weighted fixed effect model with METAL [Willer, et al. 2010]. Meta-analyses were performed in each age stratum separately, and in combined strata of all participants <25 years. Several children measured in TEST (Twins Eye Study Tasmania) and GTES (Guangzhou Twin Eye Study) had follow up measurements at an older age; therefore, only data from the oldest age were used in the combined analysis. In the Asian studies the following SNPs were excluded due to low minor allele frequency (MAF) <0.05 in the Chinese population: rs17428076, rs1656404, rs14165, rs13091182, rs12205363, rs11145465, rs10882165, and rs17183295.

Pathway analysis

Loci with significant effects ($P < 0.05$) were further explored to identify differences in effect of early-onset genes (significant loci identified in groups <10 years, 10-25 years or the combined analysis) and late-onset genes (adult subjects). Data were analysed through the use of QIAGEN's Ingenuity®.

Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) and the online software tool Database for Annotation, Visualization and Integrated Discovery (DAVID) [Huang da, et al. 2009a; Huang da, et al. 2009b].

RESULTS

Our study sample of children <10 years comprised 5,490 participants derived from 5 studies; one of European ancestry (TEST), three of Asian ancestry (SCORM, STARS, and Guangzhou Twins), and one of mixed European, African, and Asian ancestry (Generation R). Our sample of individuals aged 10-25 years included 5,000 participants derived from 6 studies; 4 of European ancestry (TEST, ALSPAC, BATS and RAINE), and 2 of Asian (STARS, Guangzhou Twins) ancestry. Our sample of adults >25 years comprised 16,274 participants derived from 10 studies; 9 of European ancestry (Croatia Split, -Kurcula and – Vis study, Gothenburg Health Study, EPIC-Norfolk and the Rotterdam Study I-III), and one Asian study (Nagahama). General characteristics per study are shown in Table I.

Genetic risk score

The genetic risk score was associated with a higher AL/CR ratio even in children aged <10 years (table II), and this association increased in magnitude with older age. Specifically, AL/CR increased with each age category from β 0.0019 (SD 0.0003) per risk allele in children <10 years, to 0.0033 (SD 0.0004) in participants aged 10-25 years, to 0.0051 (SD 0.0003) in adults (figure I). Only the adult group showed evidence for heterogeneity (heterogeneity *P*-value 0.0005) between studies, therefore, meta-analyses for this age category were also performed using the random effect model (β 0.0048; SD 0.0007; supplementary table IV). The variance explained by the genetic risk score increased from 0.7% in the children aged 6 from the Generation R study, to 3.7% for the adult participants in the RS I-III (Fig II).

Genetic loci

In children <10 years, 9/39 loci were significant at $P < 0.05$, and 3/39 were significant after correction for multiple-testing for 39 SNPs ($P < 0.00128$). The 3 loci significant after Bonferroni correction were in the vicinity of the genes *GJD2*, *ZIC2* and *CHRNA*. The 2 nominally-significant

loci with the greatest effect size (beta) were close to the *CHRNA1* and *PRSS56* genes. The other 5 loci were near *KCNQ5*, *SHISA6*, *KCNMA1*, *BMP2* and *BICC1*. Interestingly, the SNP at the *BMP2* locus had a reversed effect from that observed in adult samples, i.e., the risk allele was associated with a lower AL/CR ratio. In individuals aged 10 - 25 years, 10/39 loci showed nominally significant association with AL/CR ratio, of which 5 survived Bonferroni correction (*BMP2*, *TOX*, *KCNQ5*, *A2BP1* and *CACNA1D*). Five of the 10 SNPs above were already nominal significantly associated with AL/CR ratio in children <10 years (*GJD2*, *BICC1*, *ZIC2*, *BMP2* and *PRSS56*); of the remaining nominally-significant loci, the variant with the greatest effect in 10-25 year-olds was the SNP at the *LAMA2* locus. One variant differed significantly in effect between children <10 years and those aged 10-25 years. This was the SNP at the *BMP2* locus which, as mentioned above, showed an opposite effect to that expected in children aged <10 years (Figure III). One of the loci (*TOX*) showed evidence for heterogeneity (supplementary table III) in effect between study cohorts in the age category 10-25 years (Heterogeneity $P = 0.001$). With random effect model the effect of this SNP decreased to β 0.0062 (SE 0.0073; P 0.40)(supplementary table IV). In the combined analysis of all studies <25 years, *BICC1* and *PRSS56* reached Bonferroni adjusted significance; one additional locus (*PDE11A*) showed a nominally significant effect for AL/CR ratio. In adults, 31/39 loci showed a significant effect, of which 19/39 were Bonferroni significant. All loci, except for *ZBTB38* (β -0.0004; SE 0.0019), showed an association in the expected direction (i.e. risk allele associated with a higher AL/CR ratio). As in 10-25 years, one locus significant in adults showed evidence for heterogeneity (LOC100506035); with random effect model this locus lost statistical significance (supplementary table III and IV). Figure IV displays all estimated effect sizes per age group.

Pathway analysis

Pathway analyses were performed to gain insight into the mechanisms for early versus late-onset eye growth and myopia development. We hypothesized that loci with at least a moderate (nominally significant $P < 0.05$) effect in children and adolescents most likely had an early onset.

Hence, a locus was defined as early onset when nominally significant ($P < 0.05$) in the group <10 years of age or the group 10-25 years and no evidence for heterogeneity (in Figure IV all loci above the green line). Loci nominally significant in the adult population without a significant effect in the group <10 years of age or the group 10-25 years were grouped as late onset genes (in Figure IV all loci below the green line). We utilized two types of pathway analysis software.

Ingenuity Pathway Analysis (IPA)

IPA is a web-based software to analyse and integrate the identified SNPs based on biological functions. Analysis were performed in two separate analysis, one analysis with genes with an early onset and one analysis with late onset genes. We used the program's diseases and disorder table to identify associated diseases. Genes with an early onset in the age groups <25 years were enriched in pathways of auditory disease, organismal injury and abnormalities, and gastrointestinal disease (at FDR <5%). The genes that were significantly associated in adults predisposed to connective tissue disorders, developmental disorder (e.g. microphthalmia; with the genes *BMP4* and *SIX6*), and also gastrointestinal disease (supplementary table V).

Database for Annotation, Visualization and Integrated Discovery (DAVID)

The software program DAVID is an online knowledge database to identify overlapping functions of genes. We performed the analyses separately for early and late onset genes. Using the categories defined above, early-onset genes were significantly more than expected annotated to ion channels and ion transport. The genes annotated to these categories were *CACNA1D*, *CHRNA1*, *GJD2*, *KCNMA1* and *KCNQ5*. Late onset genes appeared to be significantly more related to neuron differentiation and visual perception. The genes involved in these categories were *RORB*, *SIX6*, *RASGRF1*, *CHD7*, *RGR*, *RDH5* and *GRIA4*. (supplementary table VI).

DISCUSSION:

This study identifies the age span during which the known GWAS-identified loci for refractive error have their greatest effect. The current meta-analysis suggests that specific loci had their

greatest effect in young children (*CHRNA1, ZIC2, KCNMA1*), while others reached the greatest effect during early teenage years (*BMP2, CACNA1D, A2BP1*). However, most appeared to have a gradual effect during the entire age span of myopia development (*LAMA2, LRRC4C, DLX1, RDH5, GRIA4, RGR, SIX6*).

Strengths of this study were the large sample size, the comparison across 3 distinct age categories, and the precision in measurements of ocular biometry. A drawback was the lack of complete cycloplegic refraction in children in several studies, which jeopardized valid measurements of RE in this age category. Thus, we used AL/CR ratio as an indicator of RE to avoid heterogeneity in the outcome. This ratio has a high correlation with RE [Hashemi, et al. 2013; Ip, et al. 2007] and was available from all studies in the consortium. Another limitation was the lack of power to detect statistically significant differences between the age groups for most genes. A pooled analysis would have increased statistical power, but raw data from individual participants were not available. Ideally, a study using longitudinal data of the same children over different age periods would have the best study design for the current analysis.

Little has been reported on the development and progression of myopia as a function of age; however, a number of studies investigated the relationship between development of ocular biometry related to age. Until the age of 25 years, corneal curvature, the crystalline lens, and axial length all evolve with age, and thereby influence refractive error. The cornea increases in radius until preschool age leading to flattening of the corneal curvature and decrease in refractive power [Augusteyn, et al. 2012]; the crystalline lens grows until 10 years of age, also reducing refractive power [Mutti, et al. 2012; Mutti, et al. 1998]. This decrease in refractive power is compensated by axial elongation which increases from 17 mm in newborns [Lim, et al. 2015] to 23.3 mm in 12-13 year olds [French, et al. 2012]. The average AL in emmetropic adults is 23.5 mm [Fotedar, et al. 2010; Gordon and Donzis 1985]. The highest growth rate of AL occurs in the first years of life and relates to emmetropisation; the growth rate after early teens is more gradual but mainly relates to myopisation [Gordon and Donzis 1985]. The exact age at

which eye growth stops is not known; generally this occurs before age 20 years, but increase in AL has been described up to the age of 25 years in university students [Fledelius 2000; Midelfart, et al. 1992].

One of the key detected GWAS-identified loci for refractive error is on chromosome 15 near the *GJD2* gene, that encodes a gap junction protein known as CX36. This protein not only processes cone-to-cone and cone-to-rod signals [Lee, et al. 2003] but also directs signaling between other retinal cells [Feigenspan, et al. 2001; Hidaka, et al. 2004]. This cell-to-cell communication appears to be under regulation of light exposure and dopamine [Bloomfield and Volgyi 2009], two factors that have an established role in eye growth and myopia development. Our data suggest that *GJD2* has an early-onset, indicating that altered retinal cell signaling, perhaps via reduced light exposure and low dopamine levels, may be a first step in myopia development. As expected, some early-onset genes also had a reported role in eye development. Knockout of *LAMA2*, a gene encoding the large extracellular glycoprotein laminin- α 2; causes growth retardation including smaller eyes with compressed cellular layers [Gupta, et al. 2012]. Mutations in the serine protease gene *PRSS56* cause a severe decrease of AL leading to microphthalmia [Nair, et al. 2011]. Another developmental gene is *ZIC2*, an enhancer-binding factor required for embryonic stem cell specification [Luo, et al. 2015]. This gene may be important for development of retinal architecture, as it is known to be involved in differentiation and proliferation of retinal progenitor cells [Watabe, et al. 2011], and development of retinal ganglion cell trajectories [Herrera, et al. 2003]. Strikingly, several other genes involved in eye development, such as *SIX6*, *CDH7*, and *DLX1*, did not show an early onset but were more significant after the age of 10 years. Other early-onset genes were ion channels such as *KCNQ5*, a potassium channel present in cone and rod photoreceptors [Zhang, et al. 2011], and *CACNA1D*, a calcium channel present in photoreceptors [Xiao, et al. 2007]. *CHRNA1* has as yet an unknown role in myopia development. It encodes the γ subunit of the embryonal

acetylcholine receptor, which is widely expressed in the retina [Hruska, et al. 1978; Hutchins and Hollyfield 1985], and is associated with multiple pterygium syndrome [Vogt, et al. 2012].

Several remarkable patterns of effect were notable. For instance, the lead SNPs at the *BMP2*, *MYO1D*, *PTPRR*, and *BMP4* loci showed an opposite effect in children <10 years than in those who were older. This is not uncommon in biology, as such a trajectory has also been described for the *FTO* locus in relation to body mass index in children [Sovio, et al. 2011]. Interestingly, gene expression studies of *BMP2* in chickens showed that mRNA of this gene in the retinal pigment epithelium is up- or down-regulated depending on the location of the image plane [Zhang, et al. 2012]. When the image was focused behind the retina, mRNA was downregulated and the vitreous chamber enlarged. This underscores a bidirectional role for *BMP2* in modulation of eye growth.

Most genes had a late onset. *BMP4* has a similar function to *BMP2* as it is also responds to optical defocus with bidirectional regulation of eye growth [Zhang, et al. 2013]. *SIX6* is a DNA-binding homeobox and has a SIX domain, which binds downstream effector molecules. It is known to influence eye size in zebrafish with knocked down *SIX6* expression [Iglesias, et al. 2014]. Other genes play a less obvious role in myopiagenesis. *MYO1D* is involved in membrane trafficking in the recycling pathway and expressed in oligodendrites [Benesh, et al. 2012]. *RORB*, a gene encoding a nuclear receptor-directing photoreceptor differentiation, is known to activate and generate S-opsin [Jia, et al. 2009; Srinivas, et al. 2006]. *DLX1* belongs to the DLX family of homeobox transcription factors, and produces GABAergic interneurons during embryonic development.

In conclusion, our study suggests that only a small proportion of the currently known GWAS-identified loci for RE exert their full effect at a young age. Furthermore, some of the pathways previously-identified by GWAS meta-analyses [Verhoeven, et al. 2013] can now be separated into early- and late-onset pathways. For example, genes coding for ion channels typically had an early onset, while genes related to connective tissue and visual feedback

mechanisms appeared to become more important at a later age. As the currently known genes play only a minor role in early-onset myopia, we question whether this type of myopia is caused by common variants in other genes, or whether rare variants with large effects determine early-onset. Future research may shed more light on genes for early-onset myopia, and unravelling these genes will open up strategies for prevention of high myopia.

Table I Participating studies and characteristics stratified per age group

Age <10 years				
Study	N	AL/CR (SD; range)	Age (SD)	Gender, % Female
STARS	207	2.99 (0.150; 2.76 – 3.46)	5.45 (2.11)	47.3
Generation R	3,874	2.87 (0.083; 2.38 – 3.90)	6.18 (0.51)	50.3
SCORM	898	3.02 (0.112; 2.63 – 3.45)	7.48 (0.87)	47.7
TEST	166	2.94 (0.101; 2.65 – 3.25)	7.53 (1.21)	52.4
GTES	345	2.97 (0.100; 2.62 – 3.45)	8.73 (0.79)	50.1
Total	5,490			
Age 10-25 years				
STARS	96	3.23 (0.127; 2.95 – 3.60)	12.23 (1.7)	58.3
GTES	699	3.13 (0.147; 2.58 – 3.82)	14.83 (1.2)	52.9
TEST	182	2.99 (0.108; 2.68 – 3.51)	15.16 (4.0)	60.4
ALSPAC	1,996	2.99 (0.099; 2.57 – 3.52)	15.46 (0.3)	53.6
BATS	983	3.03 (0.106; 2.67 – 3.82)	19.07 (3.2)	53.8
RAINE	1,044	3.05 (0.104; 2.63 – 3.54)	20.04 (0.4)	48.9
Total	5,000			
Age >25 years				
Nagahama	2,762	3.13 (0.153; 2.62 – 3.86)	52.05 (13.8)	49.0
Croatia-Split	730	3.02 (0.128; 2.38 – 3.90)	52.16 (13.0)	61.2
Croatia Korcula	832	2.99 (0.203; 2.26 – 5.73)	56.62 (13.3)	64.7
Croatia-Vis	573	2.99 (0.121; 2.50 – 3.83)	55.93 (13.8)	60.4
GHS 2	936	3.07 (0.160; 2.50 – 4.01)	59.26 (10.6)	50.0
GHS 1	1,919	3.06 (0.151; 2.30 – 3.88)	60.17 (10.7)	47.1
EPIC-Norfolk	6,051	3.05 (0.146; 2.42 – 3.95)	68.9 (8.0)	54.3
RS I-III	2,471	3.05 (0.143; 2.43 – 3.86)	70.02 (8.8)	53.6
Total	16,274			

*GTES= Guangzhou Twin Eye Study, RS I-III = Rotterdam Study I-III, GHS=Gutenberg Health Study

Table II Effect size of myopia related genes in age groups <10 years, 10-25 years, 25> years

Variant	Chr	Gene	RA	<10 years		10 - 25 years		Combined		>25 years	
				Beta (SE)	P	Beta (SE)	P	Beta (SE)	P	Beta (SE)	P
Allele Score	-	-	-	0.0019 (0.0003)	10⁻¹¹	0.0033 (0.0004)	10⁻¹⁵	0.0024 (0.0002)	10⁻²⁴	0.0051(0.0003)	10⁻⁷²
rs1652333	1	CD55	G	0.0033 (0.0017)	0.05	0.0006 (0.0024)	0.80	0.0026 (0.0014)	0.07	0.0084(0.0017)	10⁻⁶
rs4373767	1	ZC3H11B	T	0.0010 (0.0017)	0.55	0.0032 (0.0023)	0.16	0.0019 (0.0014)	0.16	0.0053(0.0017)	0.002
rs17412774	2	PABPCP2	A	0.0007 (0.0017)	0.69	0.0010 (0.0023)	0.67	0.0008 (0.0014)	0.57	0.0063(0.0017)	10⁻⁴
rs17428076	2	DLX1	C	0.0017 (0.0021)	0.43	0.0029 (0.0027)	0.28	0.0024 (0.0017)	0.16	0.0073(0.0021)	10⁻⁴
rs1898585	2	PDE11A	T	0.0022 (0.0019)	0.26	0.0050 (0.0029)	0.09	0.0034 (0.0017)	0.04	0.0057(0.0021)	0.007
rs1656404	2	PRSS56	A	0.0073 (0.0024)	0.002	0.0067 (0.0033)	0.04	0.0069 (0.0019)	10⁻⁴	0.0079(0.0024)	0.001
rs1881492	2	CHRNA1	T	0.0086 (0.0024)	10⁻⁴	0.0039 (0.0031)	0.21	0.0064 (0.0020)	0.001	0.0085(0.0022)	10⁻⁵
rs14165	3	CACNA1D	G	0.0035 (0.0020)	0.08	0.0082 (0.0026)	0.001	0.0055 (0.0016)	0.001	0.0055(0.0020)	0.005
rs13091182	3	ZBTB38	G	0.0008 (0.0020)	0.69	-0.0001 (0.0024)	0.98	0.0007 (0.0015)	0.66	-0.0004(0.0019)	0.83
rs9307551	4	LOC100506035	A	0.0007 (0.0019)	0.70	0.0037 (0.0026)	0.16	0.0020 (0.0016)	0.20	0.0051(0.0020)	0.008
rs5022942	4	BMP3	A	0.0014 (0.0018)	0.44	-0.0016 (0.0026)	0.54	0.0007 (0.0015)	0.63	0.0006(0.0020)	0.78
rs7744813	6	KCNQ5	A	0.0050 (0.0017)	0.004	0.0081 (0.0023)	10⁻⁴	0.0060 (0.0014)	10⁻⁵	0.0066(0.0018)	10⁻⁴
rs12205363	6	LAMA2	T	0.0041 (0.0041)	0.31	0.0138 (0.0046)	0.003	0.0094 (0.0031)	0.003	0.0229(0.0036)	10⁻¹⁰
rs7829127	8	ZMAT4	A	0.0025 (0.0020)	0.22	0.0019 (0.0028)	0.49	0.0025 (0.0017)	0.13	0.0072(0.0021)	0.001
rs7837791	8	TOX	G	0.0029 (0.0016)	0.06	0.0083 (0.0022)	10⁻⁴	0.0050 (0.0013)	10⁻⁴	0.0042(0.0017)	0.012
rs4237036	8	CHD7	T	0.0001 (0.0018)	0.96	0.0032 (0.0024)	0.18	0.0013 (0.0014)	0.37	0.0058(0.0018)	0.001
rs11145465	9	TJP2	A	0.0035 (0.0022)	0.11	0.0027 (0.0028)	0.33	0.0029 (0.0017)	0.09	0.0062(0.0021)	0.004
rs7042950	9	RORB	G	0.0028 (0.0019)	0.14	0.0031 (0.0026)	0.24	0.0027 (0.0016)	0.08	0.0071(0.0020)	10⁻⁴
rs7084402	10	BICC1	G	0.0035 (0.0016)	0.03	0.0066 (0.0023)	0.004	0.0050 (0.0013)	10⁻⁴	0.0074(0.0017)	10⁻⁶
rs6480859	10	KCNMA1	T	0.0040 (0.0018)	0.02	0.0037 (0.0023)	0.10	0.0040 (0.0014)	0.004	0.0015(0.0017)	0.38
rs745480	10	RGR	G	0.0007 (0.0016)	0.67	0.0021 (0.0022)	0.34	0.0011 (0.0013)	0.40	0.0055(0.0017)	0.001
rs10882165	10	CYP26A1	T	0.0012 (0.0018)	0.49	0.0002 (0.0024)	0.93	0.0007 (0.0014)	0.61	0.0011(0.0018)	0.54
rs1381566	11	LRRRC4C	G	0.0026 (0.0020)	0.21	0.0040 (0.0034)	0.23	0.0028 (0.0018)	0.12	0.0093(0.0022)	10⁻⁵
rs2155413	11	DLG2	A	0.0022 (0.0017)	0.18	0.0027 (0.0022)	0.23	0.0023 (0.0013)	0.09	0.0021(0.0017)	0.21
rs11601239	11	GRIA4	C	0.0011 (0.0016)	0.50	0.0027 (0.0022)	0.22	0.0014 (0.0013)	0.30	0.0055(0.0017)	0.001
rs3138144	12	RDH5	G	0.0020 (0.0021)	0.35	0.0039 (0.0027)	0.16	0.0028 (0.0017)	0.10	0.0045(0.0019)	0.02
rs12229663	12	PTPRR	A	-0.0023 (0.0019)	0.21	0.0046 (0.0026)	0.08	0.0000 (0.0016)	1.00	0.0069(0.0019)	10⁻⁴
rs8000973	13	ZIC2	C	0.0058 (0.0017)	10⁻⁴	0.0058 (0.0023)	0.01	0.0059 (0.0014)	10⁻⁵	0.0027(0.0017)	0.10
rs2184971	13	PCCA	A	0.0008 (0.0016)	0.61	0.0006 (0.0023)	0.80	0.0009 (0.0014)	0.48	0.0021(0.0017)	0.21
rs66913363	14	BMP4	G	-0.0025 (0.0017)	0.15	0.0040 (0.0024)	0.10	0.0006 (0.0014)	0.68	0.0047(0.0017)	0.006
rs1254319	14	SIX6	A	0.0007 (0.0017)	0.68	0.0044 (0.0024)	0.07	0.0017 (0.0014)	0.22	0.0054(0.0018)	0.002
rs524952	15	GJD2	A	0.0069 (0.0016)	10⁻⁵	0.0068 (0.0023)	0.003	0.0067 (0.0013)	10⁻⁷	0.0122(0.0016)	10⁻¹⁴
rs4778879	15	RASGRF1	G	0.0018 (0.0017)	0.29	0.0033 (0.0023)	0.15	0.0019 (0.0014)	0.17	0.0051(0.0017)	0.002
rs17648524	16	A2BP1	C	0.0018 (0.0018)	0.33	0.0079 (0.0024)	0.001	0.0039 (0.0015)	0.01	0.0077(0.0019)	10⁻⁵
rs2969180	17	SHISA6	A	0.0035 (0.0016)	0.03	0.0017 (0.0023)	0.46	0.0027 (0.0014)	0.05	0.0079(0.0017)	10⁻⁶
rs17183295	17	MYO1D	T	-0.0033 (0.0023)	0.16	0.0009 (0.0030)	0.76	-0.0018 (0.0018)	0.33	0.0089(0.0023)	10⁻⁴

rs4793501	17	KCNJ2	T	0.0029 (0.0016)	0.08	0.0001 (0.0022)	0.95	0.0019 (0.0013)	0.16	0.0041(0.0017)	0.015
rs12971120	18	CNDP2	A	0.0002 (0.0019)	0.93	0.0048 (0.0026)	0.07	0.0017 (0.0015)	0.27	0.0024(0.0019)	0.22
rs235770	20	BMP2	T	-0.0043 (0.0018)	0.02	0.0121 (0.0025)	10⁻⁶	0.0008 (0.0015)	0.60	0.0043(0.0017)	0.013

Values are betas (SE) and *P*-values, from linear regression models adjusted for sex, age and principal components if applicable meta-analysed with inversed variance meta-analysis in METAL. Bold: *P* <0.05.

Figure I. Association between genetic risk score and myopia in the three age groups

Figure II. Association between non-weighted genetic risk score and AL/CR ratio in children and adults.

Figure III. Increased effect on AL/CR ratio with age for *BMP2* gene.

Figure IV. Distribution of effects on AL/CR ratio per myopia-related gene in three age groups

REFERENCES

- Augusteyn RC, Nankivil D, Mohamed A, Maceo B, Pierre F, Parel JM. 2012. Human ocular biometry. *Exp Eye Res* 102:70-5.
- Baird PN, Schache M, Dirani M. 2010. The GENes in Myopia (GEM) study in understanding the aetiology of refractive errors. *Prog Retin Eye Res* 29(6):520-42.
- Bar Dayan Y, Levin A, Morad Y, Grotto I, Ben-David R, Goldberg A, Onn E, Avni I, Levi Y, Benyamini OG. 2005. The changing prevalence of myopia in young adults: a 13-year series of population-based prevalence surveys. *Invest Ophthalmol Vis Sci* 46(8):2760-5.
- Benesh AE, Fleming JT, Chiang C, Carter BD, Tyska MJ. 2012. Expression and localization of myosin-1d in the developing nervous system. *Brain Res* 1440:9-22.
- Bloomfield SA, Volgyi B. 2009. The diverse functional roles and regulation of neuronal gap junctions in the retina. *Nat Rev Neurosci* 10(7):495-506.
- Curtin BJ, Karlin DB. 1971. Axial length measurements and fundus changes of the myopic eye. *Am J Ophthalmol* 71(1 Pt 1):42-53.
- Farbrother JE, Kirov G, Owen MJ, Guggenheim JA. 2004. Family aggregation of high myopia: estimation of the sibling recurrence risk ratio. *Invest Ophthalmol Vis Sci* 45(9):2873-8.
- Feigenspan A, Teubner B, Willecke K, Weiler R. 2001. Expression of neuronal connexin36 in All amacrine cells of the mammalian retina. *J Neurosci* 21(1):230-9.
- Fledelius HC. 2000. Myopia profile in Copenhagen medical students 1996-98. Refractive stability over a century is suggested. *Acta Ophthalmol Scand* 78(5):501-5.
- Fotadar R, Wang JJ, Burlutsky G, Morgan IG, Rose K, Wong TY, Mitchell P. 2010. Distribution of axial length and ocular biometry measured using partial coherence laser interferometry (IOL Master) in an older white population. *Ophthalmology* 117(3):417-23.
- French AN, O'Donoghue L, Morgan IG, Saunders KJ, Mitchell P, Rose KA. 2012. Comparison of refraction and ocular biometry in European Caucasian children living in Northern Ireland and Sydney, Australia. *Invest Ophthalmol Vis Sci* 53(7):4021-31.
- Gordon RA, Donzis PB. 1985. Refractive development of the human eye. *Arch Ophthalmol* 103(6):785-9.
- Guggenheim JA, Kirov G, Hodson SA. 2000. The heritability of high myopia: a reanalysis of Goldschmidt's data. *J Med Genet* 37(3):227-31.
- Gupta VA, Kawahara G, Myers JA, Chen AT, Hall TE, Manzini MC, Currie PD, Zhou Y, Zon LI, Kunkel LM and others. 2012. A splice site mutation in laminin-alpha2 results in a severe muscular dystrophy and growth abnormalities in zebrafish. *PLoS One* 7(8):e43794.

- Hashemi H, Khabazkhoob M, Miraftab M, Emamian MH, Shariati M, Abdolahi-Nia T, Fotouhi A. 2013. Axial length to corneal radius of curvature ratio and refractive errors. *J Ophthalmic Vis Res* 8(3):220-6.
- Herrera E, Brown L, Aruga J, Rachel RA, Dolen G, Mikoshiba K, Brown S, Mason CA. 2003. Zic2 patterns binocular vision by specifying the uncrossed retinal projection. *Cell* 114(5):545-57.
- Hidaka S, Akahori Y, Kurosawa Y. 2004. Dendrodendritic electrical synapses between mammalian retinal ganglion cells. *J Neurosci* 24(46):10553-67.
- Hruska RE, White R, Azari J, Yamamura HI. 1978. Muscarinic cholinergic receptors in mammalian retina. *Brain Res* 148(2):493-8.
- Huang da W, Sherman BT, Lempicki RA. 2009a. Bioinformatics enrichment tools: paths toward the comprehensive functional analysis of large gene lists. *Nucleic Acids Res* 37(1):1-13.
- Huang da W, Sherman BT, Lempicki RA. 2009b. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protoc* 4(1):44-57.
- Hutchins JB, Hollyfield JG. 1985. Acetylcholine receptors in the human retina. *Invest Ophthalmol Vis Sci* 26(11):1550-7.
- Hysi PG, Young TL, Mackey DA, Andrew T, Fernandez-Medarde A, Solouki AM, Hewitt AW, Macgregor S, Vingerling JR, Li YJ and others. 2010. A genome-wide association study for myopia and refractive error identifies a susceptibility locus at 15q25. *Nat Genet* 42(10):902-5.
- Iglesias AI, Springelkamp H, van der Linde H, Severijnen LA, Amin N, Oostra B, Kockx CE, van den Hout MC, van Ijcken WF, Hofman A and others. 2014. Exome sequencing and functional analyses suggest that SIX6 is a gene involved in an altered proliferation-differentiation balance early in life and optic nerve degeneration at old age. *Hum Mol Genet* 23(5):1320-32.
- Ip JM, Huynh SC, Kifley A, Rose KA, Morgan IG, Varma R, Mitchell P. 2007. Variation of the contribution from axial length and other oculometric parameters to refraction by age and ethnicity. *Invest Ophthalmol Vis Sci* 48(10):4846-53.
- Jia L, Oh EC, Ng L, Srinivas M, Brooks M, Swaroop A, Forrest D. 2009. Retinoid-related orphan nuclear receptor RORbeta is an early-acting factor in rod photoreceptor development. *Proc Natl Acad Sci U S A* 106(41):17534-9.
- Kiefer AK, Tung JY, Do CB, Hinds DA, Mountain JL, Francke U, Eriksson N. 2013. Genome-wide analysis points to roles for extracellular matrix remodeling, the visual cycle, and neuronal development in myopia. *PLoS Genet* 9(2):e1003299.
- Lee EJ, Han JW, Kim HJ, Kim IB, Lee MY, Oh SJ, Chung JW, Chun MH. 2003. The immunocytochemical localization of connexin 36 at rod and cone gap junctions in the guinea pig retina. *Eur J Neurosci* 18(11):2925-34.
- Lim LS, Chua S, Tan PT, Cai S, Chong YS, Kwek K, Gluckman PD, Fortier MV, Ngo C, Qiu A and others. 2015. Eye size and shape in newborn children and their relation to axial length and refraction at 3 years. *Ophthalmic Physiol Opt* 35(4):414-23.
- Luo Z, Gao X, Lin C, Smith ER, Marshall SA, Swanson SK, Florens L, Washburn MP, Shilatifard A. 2015. Zic2 is an enhancer-binding factor required for embryonic stem cell specification. *Mol Cell* 57(4):685-94.
- McBrien NA, Gentle A. 2003. Role of the sclera in the development and pathological complications of myopia. *Prog Retin Eye Res* 22(3):307-38.
- Midelfart A, Aamo B, Sjøhaug KA, Dysthe BE. 1992. Myopia among medical students in Norway. *Acta Ophthalmol (Copenh)* 70(3):317-22.
- Mutti DO, Mitchell GL, Sinnott LT, Jones-Jordan LA, Moeschberger ML, Cotter SA, Kleinstein RN, Manny RE, Twelker JD, Zadnik K and others. 2012. Corneal and crystalline lens dimensions before and after myopia onset. *Optom Vis Sci* 89(3):251-62.

- Mutti DO, Zadnik K, Fusaro RE, Friedman NE, Sholtz RI, Adams AJ. 1998. Optical and structural development of the crystalline lens in childhood. *Invest Ophthalmol Vis Sci* 39(1):120-33.
- Nair KS, Hmani-Aifa M, Ali Z, Kearney AL, Ben Salem S, Macalinao DG, Cosma IM, Bouassida W, Hakim B, Benzina Z and others. 2011. Alteration of the serine protease PRSS56 causes angle-closure glaucoma in mice and posterior microphthalmia in humans and mice. *Nat Genet* 43(6):579-84.
- Sanfilippo PG, Hewitt AW, Hammond CJ, Mackey DA. 2010. The heritability of ocular traits. *Surv Ophthalmol* 55(6):561-83.
- Saw SM. 2006. How blinding is pathological myopia? *Br J Ophthalmol* 90(5):525-6.
- Solouki AM, Verhoeven VJ, van Duijn CM, Verkerk AJ, Ikram MK, Hysi PG, Despriet DD, van Koolwijk LM, Ho L, Ramdas WD and others. 2010. A genome-wide association study identifies a susceptibility locus for refractive errors and myopia at 15q14. *Nat Genet* 42(10):897-901.
- Sovio U, Mook-Kanamori DO, Warrington NM, Lawrence R, Briollais L, Palmer CN, Cecil J, Sandling JK, Syvanen AC, Kaakinen M and others. 2011. Association between common variation at the FTO locus and changes in body mass index from infancy to late childhood: the complex nature of genetic association through growth and development. *PLoS Genet* 7(2):e1001307.
- Srinivas M, Ng L, Liu H, Jia L, Forrest D. 2006. Activation of the blue opsin gene in cone photoreceptor development by retinoid-related orphan receptor beta. *Mol Endocrinol* 20(8):1728-41.
- Verhoeven VJ, Hysi PG, Wojciechowski R, Fan Q, Guggenheim JA, Hohn R, MacGregor S, Hewitt AW, Nag A, Cheng CY and others. 2013. Genome-wide meta-analyses of multi-ancestry cohorts identify multiple new susceptibility loci for refractive error and myopia. *Nat Genet* 45(3):314-8.
- Vitale S, Sperduto RD, Ferris FL, 3rd. 2009. Increased prevalence of myopia in the United States between 1971-1972 and 1999-2004. *Arch Ophthalmol* 127(12):1632-9.
- Vogt J, Morgan NV, Rehal P, Faivre L, Brueton LA, Becker K, Fryns JP, Holder S, Islam L, Kivuva E and others. 2012. CHRNA4 genotype-phenotype correlations in the multiple pterygium syndromes. *J Med Genet* 49(1):21-6.
- Watabe Y, Baba Y, Nakauchi H, Mizota A, Watanabe S. 2011. The role of Zic family zinc finger transcription factors in the proliferation and differentiation of retinal progenitor cells. *Biochem Biophys Res Commun* 415(1):42-7.
- Willer CJ, Li Y, Abecasis GR. 2010. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 26(17):2190-1.
- Wojciechowski R, Hysi PG. 2013. Focusing in on the complex genetics of myopia. *PLoS Genet* 9(4):e1003442.
- Xiao H, Chen X, Steele EC, Jr. 2007. Abundant L-type calcium channel Ca(v)1.3 (alpha1D) subunit mRNA is detected in rod photoreceptors of the mouse retina via in situ hybridization. *Mol Vis* 13:764-71.
- Young TL, Metlapally R, Shay AE. 2007. Complex trait genetics of refractive error. *Arch Ophthalmol* 125(1):38-48.
- Zadnik K, Manny RE, Yu JA, Mitchell GL, Cotter SA, Quiralte JC, Shipp M, Friedman NE, Kleinstein R, Walker TW and others. 2003. Ocular component data in schoolchildren as a function of age and gender. *Optom Vis Sci* 80(3):226-36.
- Zhang X, Yang D, Hughes BA. 2011. KCNQ5/K(v)7.5 potassium channel expression and subcellular localization in primate retinal pigment epithelium and neural retina. *Am J Physiol Cell Physiol* 301(5):C1017-26.
- Zhang Y, Liu Y, Ho C, Wildsoet CF. 2013. Effects of imposed defocus of opposite sign on temporal gene expression patterns of BMP4 and BMP7 in chick RPE. *Exp Eye Res* 109:98-106.
- Zhang Y, Liu Y, Wildsoet CF. 2012. Bidirectional, optical sign-dependent regulation of BMP2 gene expression in chick retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 53(10):6072-80.